Assignment¹ of the bovine *BCL2*-like 2 gene (*BCL2L2*) to BTA10q15→q21 by in situ hybridization and with somatic cell hybrids

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¹ To our knowledge, this is the first time this gene has been mapped in cattle.

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Rationale and significance

The gene *BCL2L2* encoding protein BCL2-like 2, alias BCL-W is involved in regulation of programmed cell death. It represents a member of the BCL-2 gene family comprising nearly 20 proteins, which govern the mitochondria-dependent pathway for apoptosis (Reed, 2000). BCL2-like 2 belongs to the subfamily of anti-apoptotic proteins (Denisov et al., 2003). Enforced expression of *BCL2L2* in human lymphoid and myeloid cells has been shown to contribute to reduced cell apoptosis under cytotoxic conditions (Gibson et al., 1996). Expression analysis of *BCL2L2* in Alzheimer disease brain indicates neuroprotective properties of the gene (Zhu et al., 2004). Studies of the orthologous gene in mice demonstrated an essential role in adult spermatogenesis (Ross et al., 1998). The chromosome locus for *BCL2L2* in human was found on HSA14q11.2→q12. In ruminants, the role of anti-apoptotic and pro-apoptotic genes of the BCL-2 gene family was recently examined by functional and positional analysis of five BCL-2 genes in sheep including *BCL2L2* (Lyahyai et al., 2005). Structure, function and chromosomal location of most members of the BCL-2 gene

family in cattle are unknown. Here, we report the physical mapping of bovine *BCL2L2* by FISH and PCR typing in a somatic cell hybrid panel.

Materials and methods

A partial *BCL2L2* sequence of 118 bp in length was identified by PCR in bovine genomic DNA and DNA sequencing using the primers BCL2L2-F (5'-GCCAGTCTTTTATGTTTACTCC-3') and BCL2L2-R (5'-CATAAC-CCTTCTGCCTCAGC-3') specific for the ovine *BCL2L2* gene (Acc. No. AY573197). PCR was performed using HotStar-Taq-DNA Polymerase (Qiagen) at an annealing temperature of 58 °C. PCR conditions were an initial activation of Taq-DNA polymerase at 95 °C for 15 min followed by 36 repeated cycles with 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s, and a final extension step at 72 °C for 10 min. Screening of the bovine BAC library BBI_750 (Ressource Center/Primary Database of the German Human Genome Project, <http://www.rzpd.de/>; Zhu et al., 1999) with the same set of primers revealed a genomic DNA clone. Physical mapping of *BCL2L2* was performed by PCR typing in a bovine-hamster somatic cell hybrid panel (Womack and Moll, 1986) and by FISH of the biotin-16-dUTP labeled BAC clone containing *BCL2L2* on photographed GTG-banded cattle chromosomes. The statistical analysis of PCR typing results was done according to Chevalet and Corpet (1986) and the hybridization experiment followed a standard protocol described by Pinkel et al. (1986). 200 ng probe DNA was annealed with 10 µg cattle C₀t-1 DNA and 10 µg salmon sperm DNA for 40 min at 37 °C in a pre-hybridization reaction to compete unspecific DNA sequences. The analysis of FITC fluorescence signals was done on propidium iodide-stained chromosomes.

Probe name: BBI_B750 D239

Probe type: Bovine genomic DNA-containing BAC clone

Insert size: ~ 100 kb

Vector: pBACe3.6 (BAC)

Proof of authenticity: PCR, DNA sequencing, DNA hybridization, NCBI-BLAST

Gene reference of human BCL2L2: Gibson et al. (1996)

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Fig. 1. Assignment of the *BCL2L2*-containing probe BBI_B750 D239 by FISH to BTA10. **(Left)** GTG-banded chromosomes prior to FISH; **(middle)** same chromosomes after FISH with gene specific bovine BAC clones; **(right)** locus of *BCL2L2* on BTA10. Chromosome bands correspond to the ISCNDB (2000) for GTG-banded cattle chromosomes.

Table 1. Segregation of *BCL2L2* with *BTA10* in somatic cell hybrids

Chromosome	10		
Reference marker/ <i>BCL2L2</i>	TCRA	BM888	CSSM46
+/+	12	12	12
-/-	17	16	18
+/-	0	0	0
-/+	2	3	1
Concordant:	94	90	97
Discordant:	6	10	3

Number of cells examined: 15
Number of cells with specific signal: 1 (0), 2 (2), 3 (2), 4 (10)
chromatids per cell

Mapping by FL:

Number of chromosomes examined: 26

Mean location: 0.32

Bands encompassed: 10q15→q21

Range: 73 % on 10q15 and 27 % on 10q21

Standard deviation: ± 0.022

Results

DNA sequencing of the isolated bovine BAC BBI_B750 D239 with the *BCL2L2* primers resulted in a 1096-bp genomic DNA fragment. A fragment of the bovine sequence is similar (Identities = 420/442; 95 %, Expect = 0.0) with the homologous human sequence (Acc. No. NM_004050) covering about 60 % of the human gene-coding region (in human complete exon 3; in cattle: 5'-bp position 528 to 3'-bp position 961). The sequence data have been deposited with GenBank Data Library under Acc. No. DQ001760. Performing PCR with the *BCL2L2* primers in the somatic hybrid panel, a typical bovine band was detected in 12 of the 31 hybrid cell lines. The data vector obtained was: 1000000111 1101110000 0011010000 0 (1 = present in cell line; 0 = not present in cell line). A statistical relevant concordance value of 0.97 with reference marker CSSM46 (Table 1) assigned *BCL2L2* syntenic to BTA10. Evaluation of FISH data allowed a more precise assignment of *BCL2L2* to BTA10q15→q21 (Fig. 1).

FISH Mapping data:

Most precise location: BTA10q15→q21

Nucleotide position in human chromosome reference sequence: HSA14 chromosome contig sequence NT_026437.11: ~ 4.76 Mbp

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